MALARIA SCREENING AMONG VOLUNTARY BLOOD DONORS - TO FIND OUT THE PREVALENCE - TO EVALUATE THE SENSITIVITY OF DIFFERENT TECHNIQUES

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ABBREVIATIONS

ELISA	Enzyme Linked Immunosorbent Assay
HRP-2	Histidine Rich Protein-2
PCR	Polymerase Chain Reaction
RDT	Rapid Diagnostic Test
TTI	Transfusion Transmitted Infections
TTM	Transfusion Transmitted Malaria
WHA	World Health Assembly
WHO	World Health Organization
QBC	Quantitative Buffy Coat
pLDH	Plasmodium Lactate Dehydrogenase
VBD	Voluntary Blood Donors

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1. INTRODUCTION

Voluntary blood donors constitute the safest source of blood 'contribution' to save the lives of thousands of people every day. In India, any healthy individual between the ages of 18 to 65 years can donate blood once in three months interval, maximum up to 188 times in their life time.[1] Blood Transfusion Service is a vital part of the National Health Service and there is no substitute for human blood and its components.

According to WHO, blood donation should be absolutely voluntary and free and the motive should be purely altruistic to help the unknown recipient. Blood Safety, therefore, remains an issue of major concern in transfusion medicine in developing countries where national blood transfusion services and policies, appropriate infrastructure, trained personnel and financial resources are lacking.[2] The worldwide data for the global database for blood safety of the 162 countries 39 countries are not routinely testing blood donations for one or more of the four major TTIs.[3]

The National Blood Policy in India relies heavily on voluntary blood donors, as they are usually assumed to be associated with low levels of transfusion-transmitted infections (TTIs). In India, it is mandatory to test every unit of blood collected for hepatitis B, hepatitis C, HIV, syphilis and malaria.[4] If donors test positive to any of the five infections, their blood is discarded. Voluntary blood donors consider themselves to be healthy, have no infections to their knowledge and come to the blood bank with the intention of helping someone. In order to improve the standards of Blood and its

components, the Central Govt. through Drugs Controller General of India, has formulated a comprehensive legislation to ensure better quality control system on collection, storage, testing and distribution of blood and its components. Central Govt. amended from time to time the existing requirements of Blood Banks in the Drugs & Cosmetics Act, 1940 and Rules there under to meet the latest standards.[5]

The testing for malaria parasite is mandatory as per the drugs and cosmetic act part XII B of schedule F. As per NACO, Ministry of Health and Family Welfare, Government of India guidelines 2007, test for malaria in all blood units should be tested for malarial parasites using a validated and sensitive antigen tests.[4]

According to the WHO manual 2010 for Screening Donated Blood for Transfusion-Transmissible Infections, in malarial endemic areas it is recommended to screen all blood units for malarial parasites by thick film or Serological markers to detect malarial Antibody/Antigen. The preferable method for samples with low-parasitemic load is antibody/antigen screening. This is in addition to the donor selection and deferral country-specific criteria.

Transmission of malaria by blood transfusion was one of the first recorded incidents of transfusion transmitted infection.[6] The frequency of transfusion transmitted malaria varies from 0.2 per million cases for non-endemic countries to 50 or more cases per million in endemic areas.[7]

The microscopic detection of blood though considered the gold standard for malaria diagnosis for decades, it is quite labor intensive and require adequate technical skill and man power. This had spurred the development of other microscopic malarial and rapid detection test based on the detection of malarial parasite antigen in the whole blood.[8]

There are many studies available regarding transfusion transmitted malaria and this study was undertaken to compare the gold standard microscopy with QBC and rapid diagnostic test.

2. AIM AND OBJECTIVES

To study the prevalence of malaria among voluntary blood donors.

To compare the various screening methods and to find out the most sensitive technique to screen malarial parasite among voluntary blood donors.

3. REVIEW OF LITERATURE

Blood Transfusion History

Blood transfusion has evolved over a long period from the time of Hippocrates, Where venesection was widely practiced and about one hundred years ago it became the part of routine clinical practice.[9] The description of systemic blood circulation and functions of heart and vessels by William Harvey in 1628 became a major breakthrough and a cornerstone of practice for transfusion.[10] The first animal to animal transfusion was performed by Richard Lower in the year 1666 in Oxford.[9] In 1818, James Blundell, an obstetrician at Guys and St. Thomas Hospitals in London was credited for the first human to human transfusion.[11]

In 1900, Karl Landsteiner categorized human blood groups in to A, B and C. Blood group 'C' was subsequently renamed as 'O' by Landsteiner himself. Later in the year 1902 Alfred Decastello and Adriano Sturli, students of Landsteiner found out the fourth blood group 'AB'.

For Blood transfusion, Ludvig Hektoen advocated selecting donors by blood grouping and crossmatching. Later, in 1913 the importance of crossmatching was demonstrated by Reuben Otlenberg on 128 cases of blood transfusion.

Transfusion reactions were well documented and understood by 1950s when the blood transfusion practice expanded. Major and minor reactions occurred, even after the introduction of routine typing and crossmatching.

It had been known for some time that blood transfusion transmits syphilis, malaria and smallpox. In 1942[12] , VDRL test was the first screening test to be made mandatory in U.S, since there was a strong suspicion of transmission of syphilis by blood transfusion.

It was during 1953, Paul Beeson, described transfusion transmitted hepatitis. Subsequently they found Hepatitis A, B, and C to be transmitted through blood transfusion. However, it was the outbreak of AIDS that galvanized the public attention to blood transfusion.

In the year 1985, HIV screening was made mandatory in U.S blood banks. Improved donor screening and increased testing have greatly decreased the risk of disease transmission and rendered the blood supply safer.

Blood Transfusion Safety

In 1975, 28th World Health Assembly in Geneva passed a resolution to address the issue of blood safety. The resolution WHA 28.72 urges member states for the development of national blood services on voluntary non remunerated donation of blood.[13] The aim of Blood Transfusion safety is to ensure that everyone has access to blood and blood products which are as safe as possible, adequate to meet the need of patients, available at reasonable cost, transfused only when necessary and provides us a sustainable blood program within in the health care system.[14]

Current status of Global Health safety

The World Health Organization global database on blood safety was set up in 1998 to collect and analyze data from all countries regarding blood and blood product safety on the basis of effective action to improve the blood transfusion services globally.[3]

Based on the reports from 173 countries[15], around ninety three million blood donations are donated annually, among them 50% of them are collected in the developed countries. The average donation rate in developed countries is 45.4 per 1000 population in comparison to the transitional and developing countries where 10.1 and 3.6 per 1000 population respectively.

The disease transmission depends on the type of donor, their behavior and risk status.

The following are the various types of donors:

1. VOLUNTARY DONORS

A person who gives blood, plasma or other blood components of his /her own free will and receives no payment for it, either in the form of cash or in kind which could be considered a substitute for money, this includes time off work other than reasonably needed for the donation and travel. Small tokens, refreshments and reimbursement of the direct travel costs are compatible with voluntary non-remunerated blood donation. He is an altruistic donor, who gives blood fully and willingly.[2]

CATEGORIES OF VOLUNTARY BLOOD DONOR:

a) New voluntary donor:

A Voluntary non-remunerated blood donor who has never donated blood before

b) Lapsed voluntary donor: first write regular donor and then lapsed

A Voluntary non- remunerated blood donor who has given blood in the past but does not fulfill the criteria for a regular donor

c) Regular voluntary donor :

A Voluntary non-remunerated blood donor who has donated at least three times, the last donation being within the previous year and continuous to donate regularly at least once per year.

2. OTHER CATEGORIES OF BLOOD DONORS

1) FAMILY / REPLACEMENT BLOOD DONOR[2]:

a) A donor who gives blood when it is required by a member of the patients' family or community. This may involve a hidden paid donation system in which the donor is paid by the patients' family.

b) A family / replacement donor is one who gives blood when it is required by a member of his/her family or community. This often involves concern and or payment which compromise the safety of the blood.

c) A member of the family or a friend of the patient who donates blood in replacement of blood needed for the particular patient without involvement of any monetary or other benefits from any source.

2) PAID / PROFESSIONAL DONOR:

A donor who donates blood in exchange of money or other forms of payment is called a professional donor. It is banned in India from 1st Jan 1998.[2]

Voluntary blood donors are the cornerstone of a safe and adequate supply of blood and blood products. The safest blood donors are voluntary, non-remunerated. Despite this notion, family / replacement donors still provide more than 45% of blood collected in India.[2]

Many reports confirm that paid donors and replacement donors have a higher prevalence of TTI. In a study conducted by Gupta et al (New Delhi) in 2006 among armed forces, seropositivity of HIV, anti-HCV, HbsAg show an increase in reactivity rate in replacement donor compared to voluntary donors.[16]

For a safe blood service in our country, where comprehensive test are neither possible nor pragmatic, it is best to switch over to 100% voluntary blood donation which provides the safest blood.

Transfusion Transmitted Infections

Viral Infection

The transmission of viral agent through blood transfusion was reported in 1943.[17, 18] Important transfusion transmitted viruses are Human Immunodeficiency Viruses Type 1 and Type 2, Hepatitis B, Hepatitis C, Human T-Cell Lymphotrophic Virus Type I and Type II. Other viruses include Epstein – Barr virus, Parvovirus B19, Westnile virus, GB virus C can also be transmitted through blood products[19]. The risk of transfusion transmitted viral infection has been reduced to minimal level in developed countries, but in developing countries the risk is very higher.[20]

Parasitic infection

The transmission of parasitic infection (malarial parasite) through blood transfusion was reported in 1911.Later, Babesia microti and Trypanosoma cruzi were found to be transmitted through blood transfusion. Babesiosis and Chagas disease show an increased threat to donors in developed countries. However, transfusion transmitted malaria is a major problem in developing countries.[21]

Emerging Infections

The American Association of Blood Bank has recently identified 68 infectious diseases that can be transmitted by transfusion.[22] In spite of the major transfusion transmitted infections such as HIV, Hepatitis C and Hepatitis B, other novel agents such

as prion proteins, which are responsible for prion disease or spongiform encephalopathy, have been recently recognized.[23]

The risk of transfusion transmitted infection to the recipients is declining, both in developed and developing countries. This change is due to continuing improvements in donor selection and advanced testing for infectious agents.[24]

Epidemiology of malaria

Malarial infection is a mosquito born protozoal infection of human host caused by a plasmodium parasite, commonly transmitted through the saliva of female Anopheles mosquitoes.

Five species of plasmodium genus are (P. falciparum, P. vivax, P. ovale, P. malariae and P. Knowlesi.) identified with worldwide incidence of 300-500 million cases annually with 1.2 to 2.7 billion deaths from all clinical cases reported. 90% of the cases are reported from the sub Saharan African country.

According to the World Malaria Report 2011, Malaria is considered highest geographical distributution worldwide including 106 tropical and semitropical countries with 35 countries in central Africa having the highest burden of cases and deaths.[25] Estimated annual incidences of malaria vary worldwide. Estimates of The World Malaria Report, 2011, states that 216 million malaria cases in 2010, among this approximately 81%, or 174 million cases are from the African Region[25], about 91% being due to *P.* falciparum.[25] But the actual number of cases may be much more and

among the estimated number of cases only 11% of confirmed cases were reported by national malaria control programmes.[25] Hay *et al* have estimated 451 million (95% credible interval 349-552) clinical cases of *P.falciparum* malaria in 2007.[26,27] According to the estimates of The World Malaria Report, 2011, 81% were in the African Region followed by the South-East Asia 13% and Eastern Mediterranean Regions 5%.[25] Hay *et al* reported that more than half of all estimated *P. falciparum* clinical cases occurred in Nigeria, the DRC, Myanmar (Burma) and India.[26, 27]

Figure : 1

Life cycle of malarial parasite

Malarial parasite life cycle consists of two different life cycles in two different host, sexual cycle in mosquito and asexual cycle in humans, begins when the female anopheles mosquito inoculates saliva containing malarial sporozoites in to the blood stream during the blood meal.

Asexual cycle

Exoerythrocytic phase (hepatic phase)

Sporozoites from the blood stream enter in to the liver parenchyma and develop in to hepatic schizonts. Each hepatic schizont ruptures and releases approximately 10,000-30,000 merozoites in to the blood stream. Duration of hepatic phase for P.falciparum 5.5 days ,p.vivax 8 days,p.ovale 9 days,p.malariae 15 days . In p.vivax and ovale infection some parasites remain dormant in hepatic parenchyma and causes relapses this dormant form is known as hypnozoites.

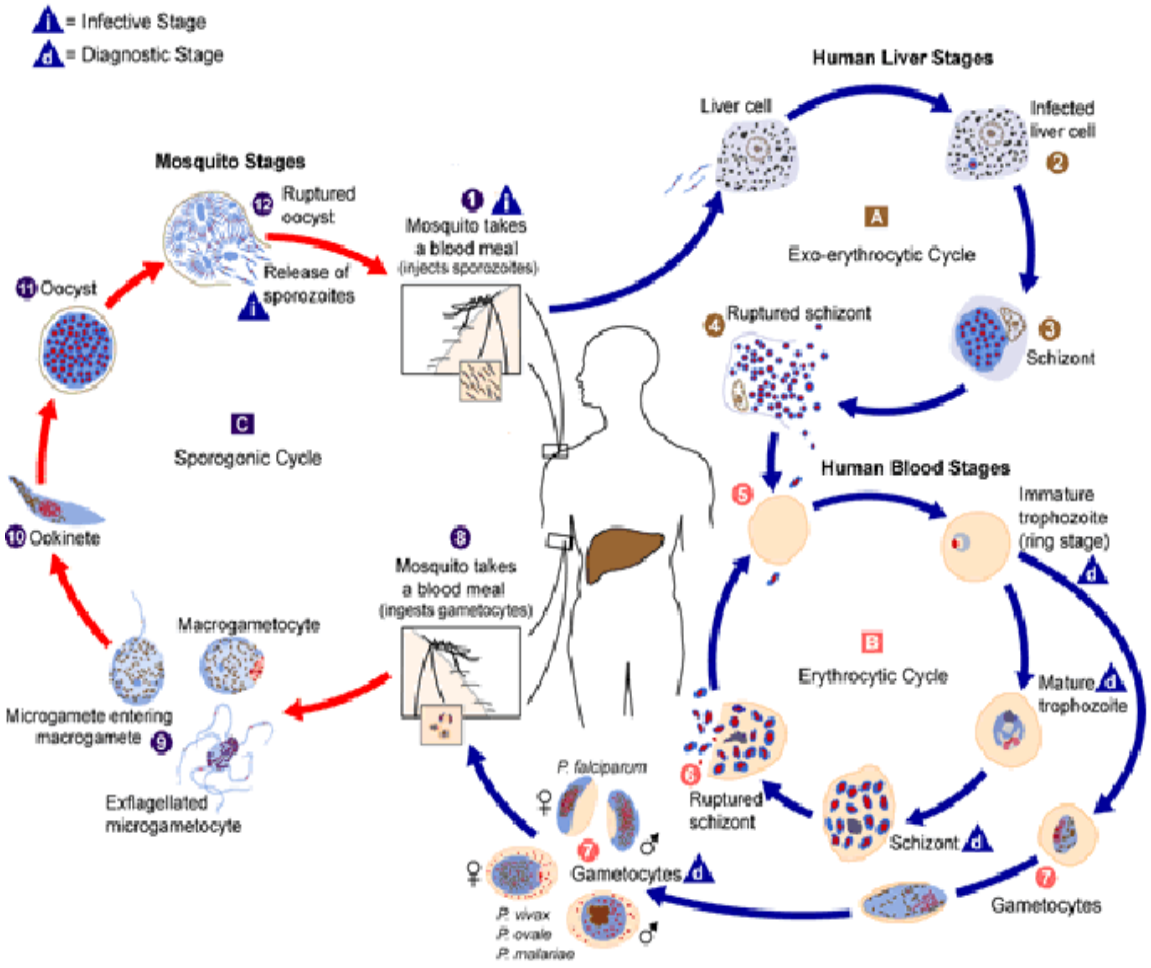
Erythrocytic phase

The merozoites from the blood stream invade the Red blood cells become trophozoites. After multiplication in the Red blood cells develop in to schizont stage around 48-72 hours. The schizont rupture and release merozoites, after multiplication of several erythrocytic cycles atleast 50 parasite/micro litre is required to produce clinical symptoms.

Sexual cycle

Few infected erythrocytes produce gametocytes other than schizonts. These gametocytes (sexual form) enter the mosquito during the blood meal and produce oocyst after sexual reproduction in the mosquito. These oocysts rupture and release sporozoites, and migrate to the salivary gland of the mosquito.[28]

Figure : 2


= Infective Stage
= Diagnostic Stage
Human Liver Stages
Liver cell
Infected liver cell
Exo-erythrocytic Cycle
Ruptured schizont
Schizont
Mosquito Stages
Ruptured oocyst
Release of sporozoites
Mosquito takes a blood meal (injects sporozoites)
Oocyst
Sporogonic Cycle
Human Blood Stages
Immature trophozoite (ring stage)
Mature trophozoite
Erythrocytic Cycle
Ookinete
Mosquito takes a blood meal (ingests gametocytes)
Macrogametocyte
Microgamete entering macrogamete
Exflagellated microgametocyte
P. falciparum
Ruptured schizont
Schizont
Gametocytes
Gametocytes
P. vivax
P. ovale
P. malariae

In endemic areas asymptomatic P.vivax carrier-state is possible because of low-parasitemic load and semi-immune status of the host. The asymptomatic individual can show recrudescence of infection in days or weeks or sometimes in months after initial infection. P.vivax sporozoites in hepatocytes are known to remain dormant as hypnozoites, which can result in relapse after many months to years of asymptomatic carrier-state.

Malaria transmission

Transmission of malaria parasites primarily occurs through the bite of the blood feeding female anopheles mosquito. Besides transmission by mosquitoes, direct inoculation is another well documented mode of acquiring malaria. Direct inoculation has resulted in malaria after accidental exposure in healthcare workers, among injecting drug users sharing used needles and in blood transfusion.[29, 30]

Malaria transmission by transfusion is the most common form of direct inoculation.

A review in 2005 found 22 published cases of occupational *P. falciparum* malaria following accidental blood exposure.[31] All the cases were from non-endemic countries. Accidental exposure to infected blood may be challenging to diagnose in areas where infections are not endemic[32] but with increasing travel to malaria endemic areas and the possibility of more patients being admitted and treated for malaria, this route may become more important in occupational health.[31]

Other modes of transmission

1. ***Mother to the growing fetus*** (Congenital malaria): Transfer of parasitized red cells from infected mother to the child either transplacentally or during labor can lead to malaria in the newborn, which is known as congenital malaria.[33]

2. ***Needle stick injury:*** Cases of malaria transmission through needle-stick injuries occur accidentally among health care professionals and also due to needle sharing among drug addicts.[34, 35]

3. ***Transfusion Transmitted Malaria:*** Malaria can be transmitted by transfusion of blood from infected donors and was first reported in 1911.[36] One of the most common transfusion-transmitted infections to be prevented is transfusion malaria.[34, 35] The risk of acquiring transfusion malaria is very low (1 case per 4 million) in non endemic countries such as the United States, whereas in the endemic countries, it is much higher (>50 cases per million donor units). [35, 37]

After subsequent malaria infection, the individual may remain infective for weeks to months, or even years, in case of *P. malariae* infection and proven carriers of *P. malariae* should never donate blood. The transmission risk is elevated in transfusion of fresh, whole blood, particularly when the blood has been stored for less than 5 days and the risk is significantly lesser after 2 weeks.[38]

In case of transfusions of plasma, plasma components, or derivatives devoid of intact red cells the risk of transmission is extremely low.[39] Most donors implicated in transfusion-transmitted malaria are predominantly semi-immune with very low parasite loads and the infectious dose is estimated to be 1 to 10 parasites in a unit of blood. Hence, it is difficult to identify malarial infection in donated blood specimens. Detection of such low parasitemia is difficult or impossible with the peripheral smear examination or even with more sensitive tests such as the antigen or polymerase chain reaction (PCR) assays in few occasions.[6,40,41,42]

Although universal serological screening in non endemic regions is not cost-effective, targeted screening of donors identified at risk by travel-based questioning can significantly reduce wastage.[38] The development of automated protein microarray-based technology has the potential to further enhance antibody/antigen sensitivity.[38] There are reports of successful use of Rapid Diagnostic Tests in screening donated blood.[8]

In non-endemic countries, donor deferral in combination with screening for specific antimalarial immunoglobulin provides an effective means of minimizing the risk of malarial transmission. In addition, the administration of antimalarials to transfusion recipients may help to prevent transmission.[6] In endemic regions due to high risk of transfusion malaria, it is also important to ensure that blood collected in highly endemic regions is not transfused to patients from areas of low endemicity.[40] Chemoprophylaxis was found to be particularly useful in endemic countries for

protecting young children with no or little malarial immunity from developing acute and potentially fatal post transfusion malaria[42].

Transfusion malaria manifests with a shorter incubation period of 2–4 days as the inoculum contains the erythrocytic forms of the parasite and pre-erthrocytic phase of the life cycle within the liver does not occur. Typical symptoms of fever, malaise, and headache occur and in case of *P. falciparum* infection, particularly in the non immune patients, the infection can progress rapidly into fatal illness.

Diagnosis of transfusion malaria requires a keen sense of clinical suspicion and any recipient of blood transfusion developing such symptoms should be tested immediately for malaria. As the transfusion-acquired *P. vivax* malaria does not have the exo- erythrocytic phase, relapses do not occur.[34, 35,37,43,44]

TTM in endemic countries

Transfusion transmitted malaria is very difficult to differentiate from natural infection due to the endemicity of malaria .Many donors and patients in endemic areas are already infected or at high risk of malarial infection. Mohamed Siddig M. Ali *et al* in their study in Sudan found that the prevalence of post transfusion malaria in transfusion recipients was 3.5%, four days after transfusion.[45]

All patients in the study (twelve) who received malaria positive blood screened by peripheral blood smear study became malaria positive. There were also two patients who did not receive malaria positive blood but still developed malarial parasitemia 4

days after transfusion. In malaria endemic countries post transfusion malaria cannot be equated to TTM because there are other sources of transmission. Naheed Ali *et al* in their study concluded TTM is a risk in malaria endemic country like Pakistan.[46]

Anju Dubey *et al* in their study on seroprevalence of malaria in blood donors and multitransfused patients in northern India concluded that malaria antibody prevalence in blood donors was high and blood units donated by such donors have high risk potential and special processing may be undertaken to reduce the risk of TTM.[47]

Okocha *et al* in their study on the prevalence of malaria parasitemia in blood donors in a Nigerian teaching hospital found that one in three blood transfusions carries the risk of transmitting malaria parasite to the recipient.[48]

TTM in non-endemic countries

Most of the data are of TTM from non-endemic countries for Malaria; this may be due to the ease of diagnosis in non-endemic countries compared to endemic countries where it cannot be proven whether malaria developed after post transfusion or from a reinfection or recrudescence.

There were about 350 cases of TTM reported between 1911 and 1950.[37] During the period of 1950 to 1972, 1756 cases had been reported in 49 countries.

As transfusion of blood and its components increased, more cases of TTM were expected. On the other hand with an increased awareness of this problem, more

interventions have been introduced to reduce its occurrence including screening test and deferral of donors who have travelled to malaria endemic countries.

In a study by SHOT, two confirmed cases of TTM have been reported from 1996 till 2010 in UK[49]. In Japan, twenty two cases of TTM have been reported since 1945.[50] Three cases of TTM were reported in 2009 in US.[51] Garraud *et al* in their study from 1990-2006 in France found that there was one case of TTM reported for every three years.[52]

SEROPREVALENCE OF TRANSFUSION TRANSMITTED MALARIA

Table : 1

I. IN ENDEMIC COUNTRIES

Author	Country	Malaria Study Group	Malaria Antigen Prevalence
Diop *et al*[55]	Senegal	Blood donors	0.53%
Saeed *et al*[56]	Saudi Arabia	Blood donors	0.17%
Lim CS *et al*[57]	Korea	Blood donors	1.7%
Choudhury N *et al*[58]	India	Blood donors	0.35%
Anju Dubey *et al*[47]	India	Blood donors	0.09%
Bahadur *et al*[8]	India	Blood donors	0.03%

Table : 2

II. IN NON-ENDEMIC COUNTRIES

Author	Country	Malaria Study Group	Malaria Antibody Prevalence
Chiodini *et al*[6]	UK	Blood Donors	0.45%
Soler *et al*[53]	France	Blood Donors	1.62%
Seed *et al*[54]	Australia	Malaria Exposed blood donors	1.33%

Saeed *et al* in their study at Saudi Arabia found the malaria prevalence rate among voluntary blood donors by antigen detection method by ELISA technique was 0.17%. [56]

Diop *et al* in their study at Senegal found the malaria prevalence rate among blood donors by antigen detection method by ELISA technique was 0.53%.[55]

Table : 3

Incubation period for TTM

Falciparum	Vivax	Malaria	Ovale
7-13 days	8-12 days	24-28 days	12-14 days

Parasite Viability in stored blood

Plasmodium Parasites can survive in the refrigeration for up to 18 – 20 days at 4degrees C. [6, 59] There were 7.1 fold reductions after 14 days but parasites remain detectable by microscopy for 28 days when the blood is stored at 4 degree C. Parasites did not show in vitro replication when cultured after 14 days storage. This shows that the viability of the parasite is reduced after prolonged refrigeration.

PREVENTION STRATEGY FOR TTM

Screening test

Direct and indirect methods are used as screening tests in the diagnosis of malaria Direct method is detection of antigen and indirect method is detection of antibody.

Direct Method

1) Peripheral smear
2) QBC Quantitative Buffy coat method
3) Immunochromatography / rapid test method

Indirect Method

1) Enzyme-Linked Immunosorbent Assay
2) Indirect Fluorescent Antibody Test
3) Polymerase Chain Reaction

Peripheral Smear

Two types of blood film preparation are used thick and thin**.**

Thick smear is for identification of parasite Thin smear is for identification of species,

For examination of thick film, 100 microscope fields should be looked at.[60] Microscopy has a limit of detection of 20 – 30 parasites per µl of blood and low parasiaemia can be missed by microscopists. [61, 62] Accurate determination of parasite density is done by thin film by counting the parasites against at least 100,000 red blood cells.[63] The stains that are used for peripheral smear are Giemsa and Leishman. Microscopy remains the gold standard for malaria diagnosis.

Malaria Rapid Diagnostic Test

Immunochromotographic or rapid diagnostic test is based on the capture of malarial antigens. Different formats are commercially available Dip Sticks, Cards, and Cassettes. The principle is same for all. Immunochromotographic procedures utilize monoclonal or polyclonal antibodies which are conjugated to either gold particles or liposome's containing selenium dye. It is used to capture malaria antigen. The migration of liquid across the nitro cellulose membrane allows capturing the malaria antigen to get bound to a second immobilized monoclonal antibody and produce the visible colored line.[64]

There are two types of malarial antigens, histidine-rich protein -2 (HRP-2) and parasite lactate dehydrogenase pLDH. Plasmodia aldolase is another antigen introduced few years ago.[65] It is found in all species of plasmodium.[66]

Histidine Rich protein-2 (HRP-2)

HRP-2 is seen only in P.falciparum [hospital care for children] and kits with antibody against HRP-2 has got sensitivity of 85% and specificity of 100%.[67 - 74] As HRP-2 is present only in P.falciparum, antibody against HRP-2 cannot be used for the detection of other human malaria parasite such as P. vivax, P. ovale malaria.[75] HRP-2 persists in the blood stream after malarial episode so it cannot be used for anti-malarial therapy monitoring.[76]

Plasmodium Lactate Dehydrogenase (pLDH)

The Plasmodium enzyme pLDH is produced by malaria parasite during the growth in Red Cells. All the four human malarial parasites produce a unique isomer of pLDH activity[77]

Test for P.vivax is based on LDH and it has recently been developed and evaluated in the Republic of Korea[78] in which P.vivax accounts for 99.9% of all malaria cases.[79,80] The pLDH becomes negative soon after the parasite clearance.

Sensitivity and Specificity of RDTs

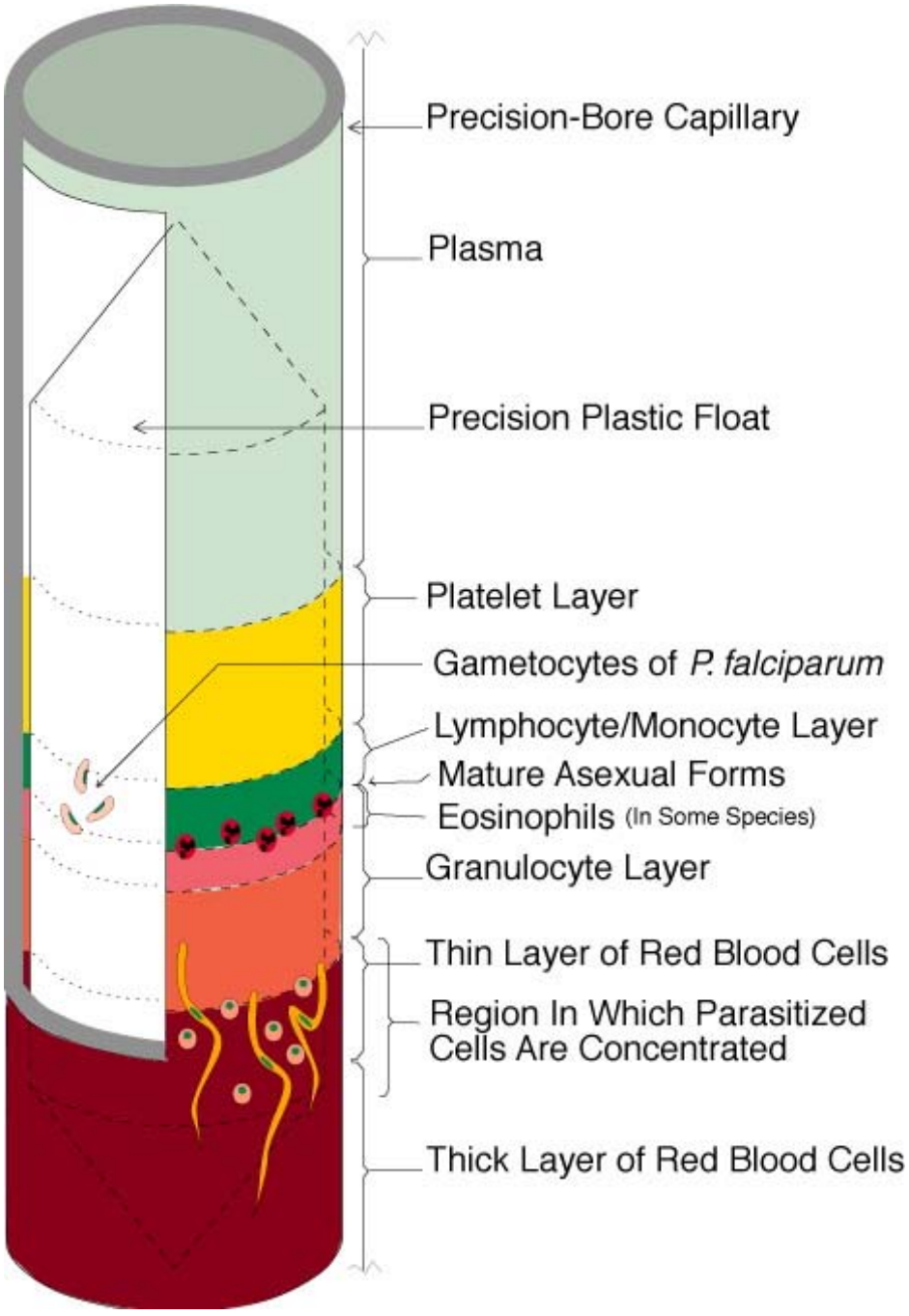
RDTs have 88 – 100% sensitivity and 92-95% specificity for the detection of P.falciparum.[81] The RDTs sensitivity decreases when the parasite density is less than 100 parasites / µl.[82] RDTs are not a replacement for microscopy but where microscopy is not possible RDTs should be introduced.[83]

RDTs have been used since early 1990s and they can perform with high accuracy.[82] A new generation of 3 band RDTs use P.aldolase , pan malarial antigen(pLDH), in addition to HRP-2 to identify non-falciparum infections.[84] This will distinguish P.falciparum from other species but it cannot differentiate between other non falciparum species.

QBC Test principle

The QBC analysis utilize the density gradient layering of stained blood cells with the expansion of micro hematocrit buffycoat .The QBC technology is less dense, parasite infected cells concentrated within the separated red cells white cells layers of the spun QBC tube. QBC tube is internally coated with acrydine orange stain and anti-coagulants .The main advantage of the acrydine orange dye is to visualize the malaria parasites. The centrifugally separated cell layers is achieved with plastic float ,inserted to the tube after filling the capillary or Venus blade and ceiling with the plastic closure the separated layers are clearly identifiable in the spun tube or dark and light red cells , granulocytes – lymphocytes – monocytes and platelets. Examining under the fluorescent microscope of the interface region of the right red cells and granulocytes permits the rapid and unambiguous detection of the stained malaria organ.

Figure : 3


Precision-Bore Capillary
Plasma
Precision Plastic Float
Platelet Layer
Gametocytes of P. falciparum
Lymphocyte/Monocyte Layer
Mature Asexual Forms
Eosinophils (In Some Species)
Granulocyte Layer
Thin Layer of Red Blood Cells
Region In Which Parasitized Cells Are Concentrated
Thick Layer of Red Blood Cells

Antigen detection by ELISA

Enzyme linked immunoassays have been developed that are capable of detecting malaria antigens.[86, 87, 88] The antigen which is being detected is HRP-2 same which is used for RDTs. In this technique, wells are coated with monoclonal antibodies against HRP-2 of P. falciparum.[89]

Antigen ELISA has been recommended for evaluation in blood banks but surprisingly, there are not many published studies. A Recent study which was conducted among blood donors in Venezuela reported the sensitivity and specificity was very high 100% and 98% respectively.[90]

Polymerase chain reaction

The development of malaria nucleic acid amplification techniques has been based on the polymerase chain reaction method. PCR is a method which is used to amplify nucleic acid sequences of the malaria parasite which may be present in blood. Following PCR the amplification product can be detected using gel electrophoresis followed by ethidium bromide staining. Based on various modifications, different types of PCR methods available including multiplex PCR, nested PCR and real time PCR.

PCR is the most sensitive and specific method to detect malaria parasites.[91] Sensitive PCR techniques have been developed that are capable of detecting parasites at a density of one per microlitre in contrast with 10-50 parasites per microlitre ,which is the threshold of an experienced microscopist.[92] Such a highly sensitive and specific test would be ideal especially as low parasitemia is expected in donors but PCR requires advanced infrastructure is required for training personnel, quality control ,maintaining equipment and preventing contamination.[84] Such a high cost will be unaffordable for many malaria endemic countries that are also developing countries. It is unlikely that PCR will be suitable for routine purposes in the near future[93] although it may become more accessible in the longer term.

Anti Malarial in blood bags

The in vitro processing of donor blood with anti-malarial in blood bags to kill parasites before the blood is transfused has been suggested as an additional measure to prevent TTM. Two anti-malarial, quinine and sulfadoxinepyrimethamine (SP) have been used experimentally and found to effective against parasites in blood bags. [94, 95] In a study in Sudan, SP for example at a dose of 179.65 μg/L killed 99% of all parasites within 24 hours parasites. There are however concern that such an intervention can contaminate the blood bag and has the potential to increase the spread of resistance to the particular anti-malarial.[96] In vitro processing of donor blood with anti-malarial has remained an experimental intervention and has not been used in blood for patients.

Medication to blood recipients

Presumptive treatment of transfusion recipients in malaria endemic countries is recommended by the WHO[97] and countries such as Kenya and Senegal have implemented this recommendation. There are only few data on the extent to which antimalarials are currently being used as presumptive treatment. In the past administration of antimalarials such as chloroquine to recipients of blood transfusion was in wide practice. Resistant to chloroquine has largely replaced more expensive artemisinin based therapy. This has increased 5-7 fold the cost of treating all transfusion recipients with antimalarials making this practice unaffordable on a wide scale.

4. MATERIALS AND METHODS

A cross sectional study was conducted to study the prevalence of malaria among voluntary blood donors. Voluntary Blood Donors (VBD) was recruited from the blood bank of the department of Transfusion medicine and Immunohematology of the Tamil Nadu Dr.M.G.R.Medical University, Chennai, Tamil Nadu. All the study participants were selected by the following selection criteria.

INCLUSION CRITERIA:

Voluntary Blood Donors as per the eligibility criteria given in the Technical Manual of the Directorate General of Health Services.

EXCLUSION CRITERIA:

Voluntary blood donors - not willing to participate in the study.

ESTIMATED SAMPLE SIZE

Sample Size was calculated by

$$N= Z^2 P (1-P) / d^2$$

Where N = Sample Size

Z = Z statistic for a level of confidence.

P = expected prevalence or proportion

d = Precision

Z statistic (z): CI-95%, Z value is 1.96

N = (1.96)2 x 0.1 (1-0.1) / (0.05)2 = 144

10% prevalence the sample size is 144,

The sample size is around- 150

Laboratory Methods

2ml of EDTA blood sample was collected from the donor and used for screening malaria parasite by

1. Peripheral blood smear
2. Quantitative Buffy Coat method.
3. Rapid Card Test

Data Collection Method

After obtaining the informed consent from the donor, a semi-structured questionnaire (standard Voluntary donor form) was used to obtain information on demographic details and other risk factors such as previous history of malaria, treatment taken for malaria etc.

Peripheral Smear Method

Microscopic examination of the blood films is known as the current universal "gold standard".

Method for the diagnosis of the malaria

Preparation of thick film

A small drop of blood taken from the EDTA blood sample placed over the clean glass slide and thick blood film made with the help of another glass slide spreading it out about $1cm^2$

Surface area. The exact thickness of the blood film can be accessed by placing a piece of newspaper over the prepared blood film and the print should be visible. Allowed the film to dry at 37^0 C for 30 minutes or 50 to 60^0 C. for seven minutes.

Commercially available Leishman's stain was used to stain the thick film of peripheral smear.

1) After deheamoglobinised with distilled water the thick film is stained with Leishman stain

2) The film is kept in the rack for 30 sec to 1 Min to get fixed

3) Then buffered distilled water is added twice the amount at a pH of 7.2

4) Allowed the stain for ten minutes undisturbed

5) Washed the stain with Tap water.

Thin Blood Film

If the parasites are seen in the thick film, the thin film should be examined to determine the species. The thin film morphological identification of the parasite to species level in thin film is easier and has greater specificity when compared to thick film. This method is made as a choice for routine screening in which parasitemia is very low.

Examination of thin blood film

Parasitemia is estimated by finding the number of parasitized RBC in 10,000 RBC. The number of parasitized cells is expressed as percentage. Roughly the number of parasites present in one micro liter is calculated by assuming that one micro liter of blood contains 5 x 10^6 RBC; 1 percent of parasitemia contains 1 percent per 100 RBC.

The stained film is examined under oil immersion microscope.

Figure : 4

Quantitative Buffy Coat Method

Quantitative Buffy coat method was done with acridine orange dye which stains nucleus and cytoplasm by binding with DNA and RNA. The presence of parasite and its morphology examined under fluorescent microscopy after excited at 480 nm, the nucleus show yellowish green and the cytoplasm show bright red fluorescence. The general morphology and outline structure of the parasite are well preserved like specimens stained by the giemsa stain.Red blood cells will not emit fluorescence colour due to absence of staining by acridine orange dye.

The quantitative Buffy coat capillary tubes are available with 75mm and 1.677mm in length and diameter respectively coated with anticoagulants EDTA and heparin at the top of the tube and(fill end)and acridine orange dye with potassium oxalate at the opposite end .

The QBC tube filled with approximately 55-65 microlitres to dissolve residues in the sample QBC tubes are rotated for 10 seconds.

The plastic float with a specific gravity of 1.055 which is midway between plasma and RBC and 20mm in size is used to create 40micron space between the interior and exterior of the inserted plastic float to achieve expansion of cell layers which is centrifugally separated.

After centrifuge at 12,000g for 5 minutes the tubes are examined under oil immersion by focusing under the Buffy coat, the area around the plastic float.

The presence of malarial parasite DNA and RNA is identified by the green and orange fluorescence emitted respectively.

Every tube was examined until the presence of parasite or for maximum five minutes

Figure : 5

Figure : 6


IMG_0397 - Copy.JPG
Type: JPEG image
Size: 3.02 MB
Dimension: 4608 x 3456
pixels

Rapid Card Test

The immunochromatographic rapid kid components are used after checking the colour of reagents which is blue in colour normally if it is pink or colourless should be discarded.

The test kid ready to use after bringing it to the room temperature port A in the test kid filled with 5 microlitre of anticoagulated blood by micropipette and allowing it mix evenly in port B two drops of buffer solution delivered vertically with the help of plstic dropper.

The results are interpreted after 20 minutes. Two pink-purple bands in the Pf and Pan Region in the test window T along with control band indicate positive for plasmodium falcipaeum or mixed infection. Only one pink-purple band in control indicate negative result and one pink-purple band in Pan Region at test window T along with control indicate positive for other species

The following results indicate invalid test.

1. Absence of bands in all regions
2. Presence of bands only in test region without control band.

Figure : 7

Informed Consent Form

Study objective was explained to the participants and then informed written consent was obtained in vernacular language as per the format enclosed.

Ethical Issues

In this study, human beings were used for the study and also invasive procedures were involved. Hence, human ethical clearance was obtained from the IRB of The Tamil Nadu Dr.M.G.R.Medical University. (Copy Enclosed).

5. RESULT

A total of 250 blood donors were enrolled for this study. Of which 30 % of the donors + were in the age group of less than 22years followed by 26% who were in the age group of 26-33years. In our study malarial parasite positive donor was in the age group of less than 22 years.

Table : 3

Age Distribution of Voluntary Blood Donors

Age in years	No.of Donors	Percentage
<22	76	30.4%
22-26	52	20.8%
26-33	65	26%
>33	57	22.8%
Total	250	100%

Figure : 8

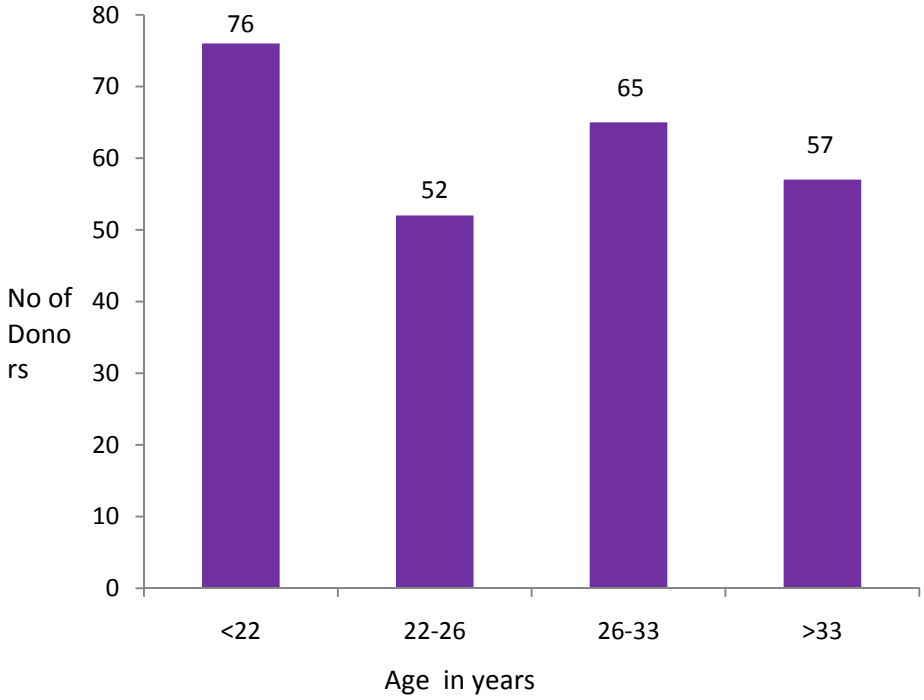
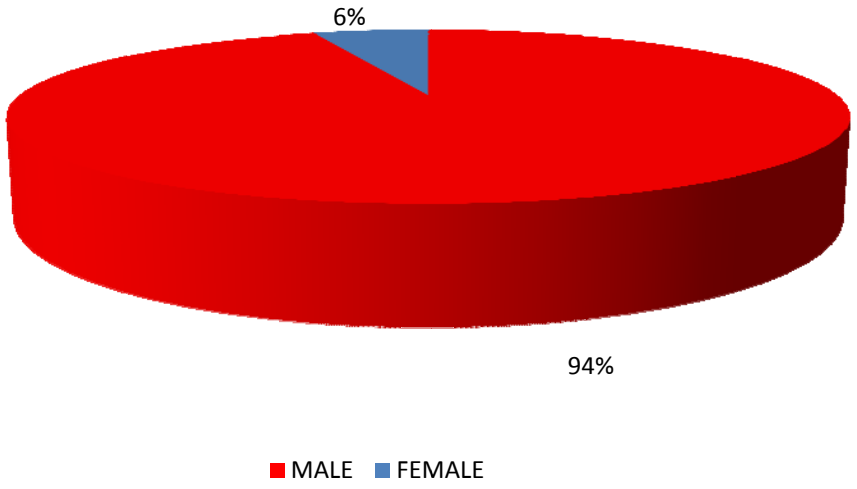

80
70
60
50
40
30
20
10
0
No of Donors
76
52
65
57
<22
22-26
26-33
>33
Age in years

Table : 4

Sex Distribution of the Voluntary Blood Donors

Sex	No.of Donors	Percentage
Male	**236**	**94%**
Female	**14**	**6%**
Total	**250**	**100%**

Figure : 9



Sex distributions of the donors were 94% males and 6% females. Among the donors male donors were high in number while compared with female donors.In our study malaria positive donor was a male donor.

Table : 5

Distribution of Age among gender

Sex	Mean age in yrs	Median (yrs)	Range(yrs)	Min (yrs)	Max (yrs)
Male	**28.49**	**27.00**	**35**	**18**	**53**
Female	**20.29**	**20.00**	**8**	**18**	**26**

The below table shows the distribution of blood group among total population recruited.

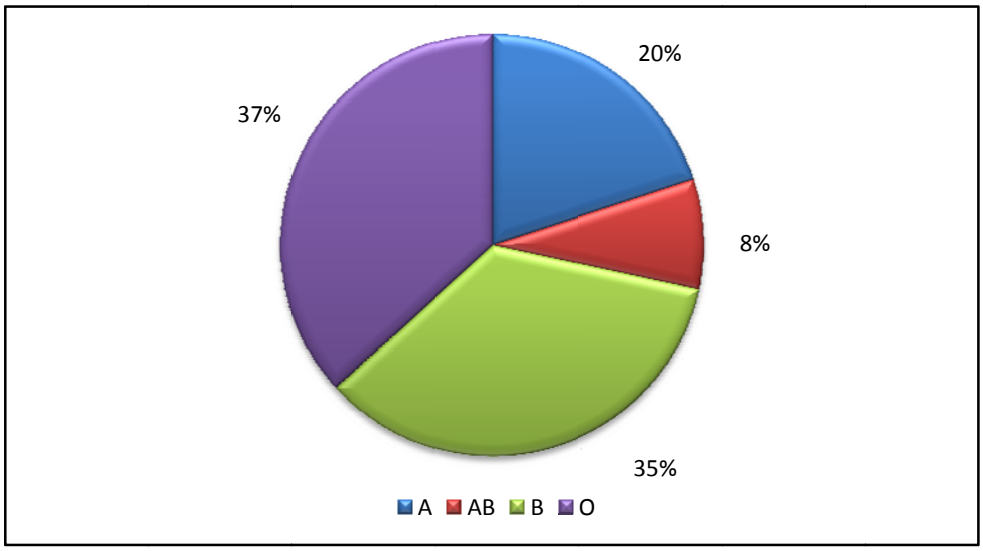
Table : 6

Blood Group Distribution of Voluntary Blood Donors

Blood Group	No.of Donors	Percentage
A	50	20%
AB	21	8%
B	87	35%
O	92	37%
Total	250	100%

Figure : 10

Distribution of blood donors in percentage



In our study, the percentage distribution of voluntary blood donors on the basis of blood groups were 37% for O group followed by 35% for B group, 20% for A group and 8% for AB group.

In this study malaria positive donor was B Blood group.

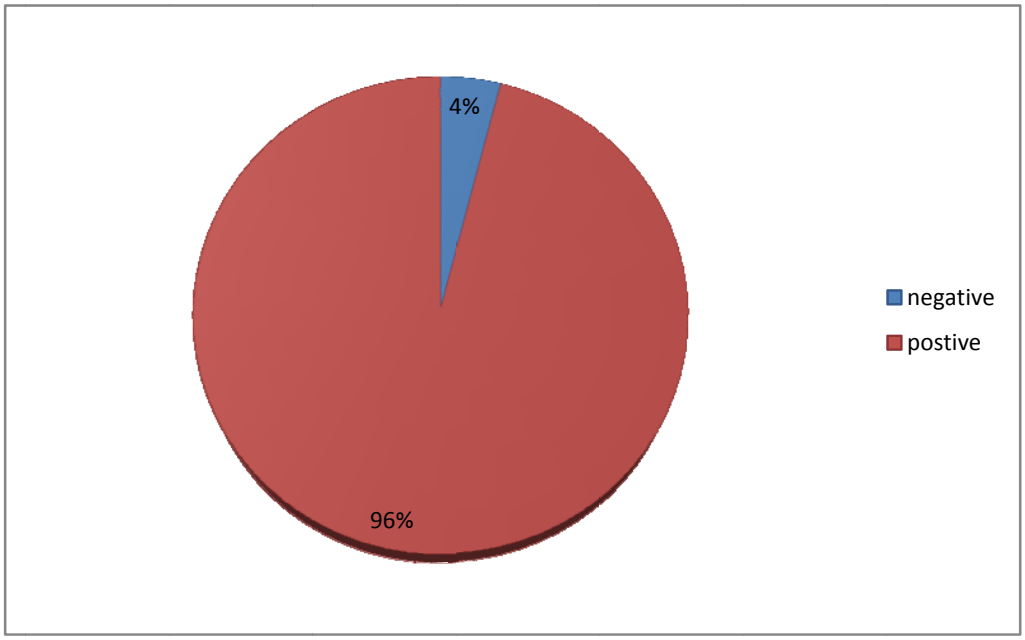
Table : 7

Distribution of Rh group in voluntary blood donors

Rh Group	No.of Donors	Percentage
Positive	240	96%
Negative	10	4%
Total	250	100%

Figure : 11

Rh distribution among blood donors



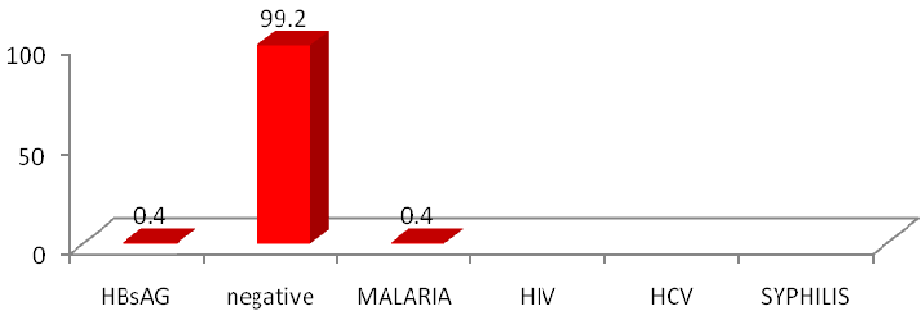
In our study, 96% of the donors were Rh positive and 4% of the donors were Rh negative. Malaria positive donor was Rh positive.

Table : 8

TTI Screening Among Voluntary Blood Donors

TTI	Postive	Negative	Total
HBsAG	1	249	250
Malaria	1	249	250
HIV	0	250	250
HCV	0	250	250
VDRL	0	250	250

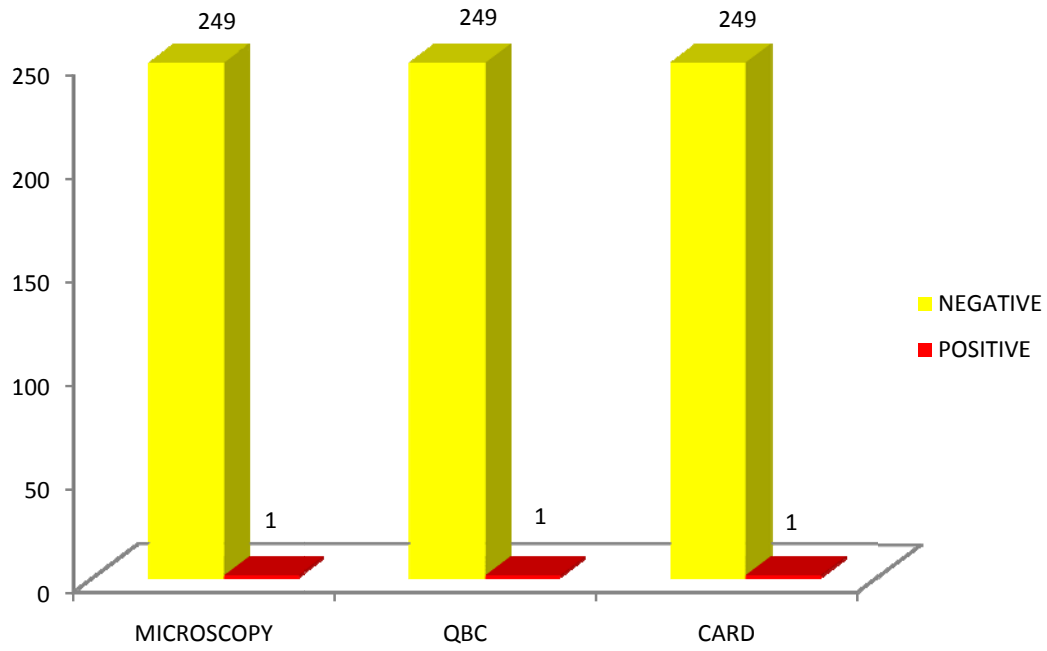
Figure : 12



In our study there was 1 donor positive for HBsAg which was 0.4% and there was another donor positive for malaria in the TTI screening which was 0.4% and there were no positivity for HIV, HCV and Syphilis.

Figure : 13

Comparative of Microscopy, QBC and Card Test for Malaria Screening among Voluntary Blood Donors

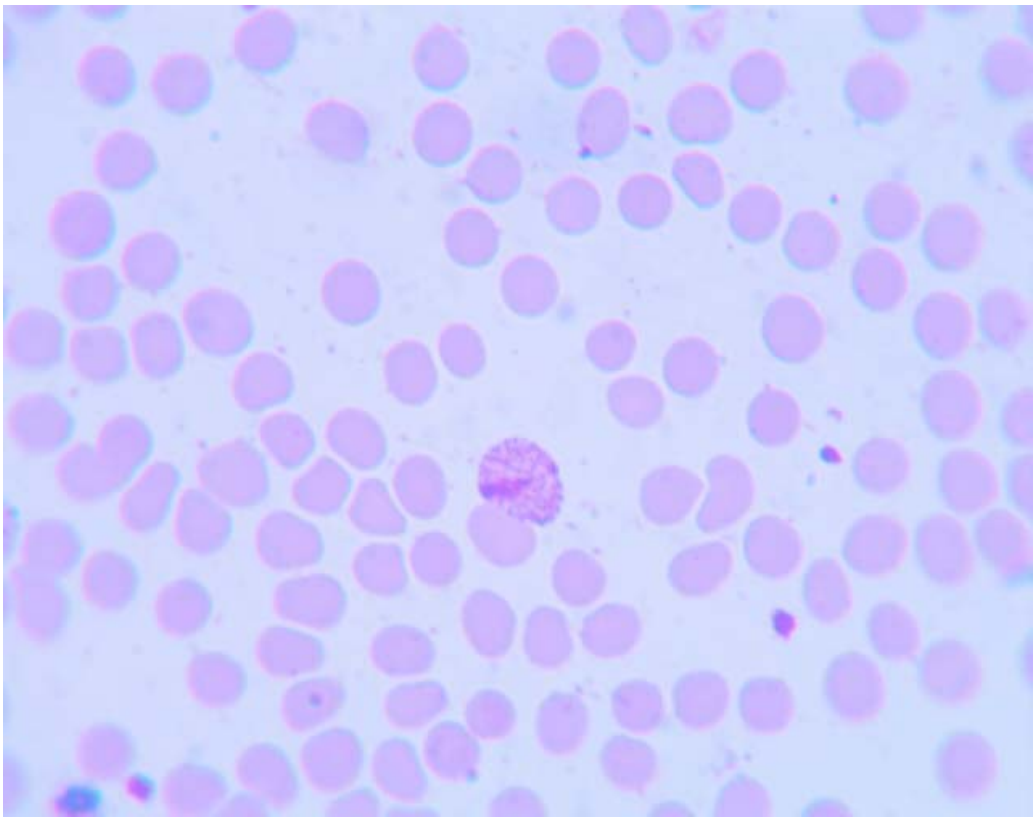


In our study of the 250 donors, there was 1 donor postive for malaria which was identified by Microscopy,Rapid Card and QBC methods.The species of malarial parasite identified by the above methods was plasmodium vivax.

MICROSCOPY FOR MALRIAL PARASITE SCREENING

Malarial parasite was observed under microscope by using thick and thin film. P.vivax gametocyte stage was observed under the microscope

Figure : 14



Quantitative Buffy Coat Method

By this method also the same type of parasite was observed as showed in the picture below.

Figure : 15

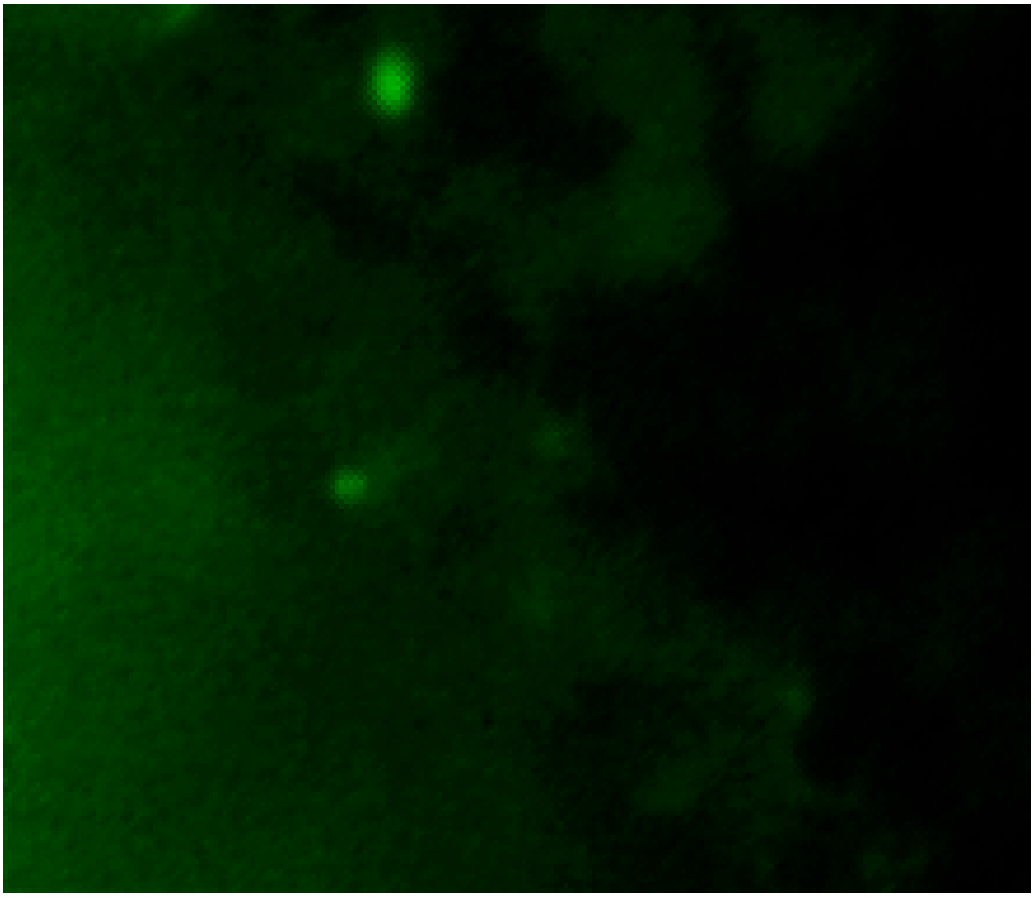


Figure : 16

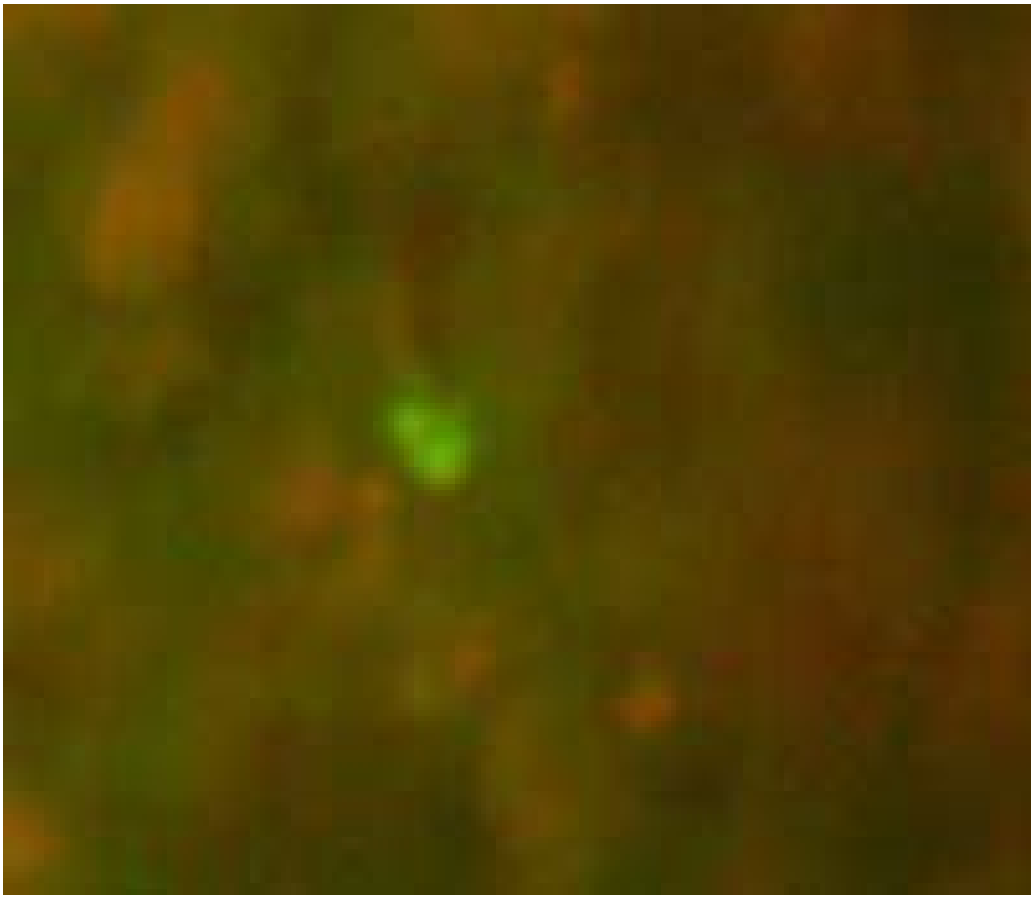
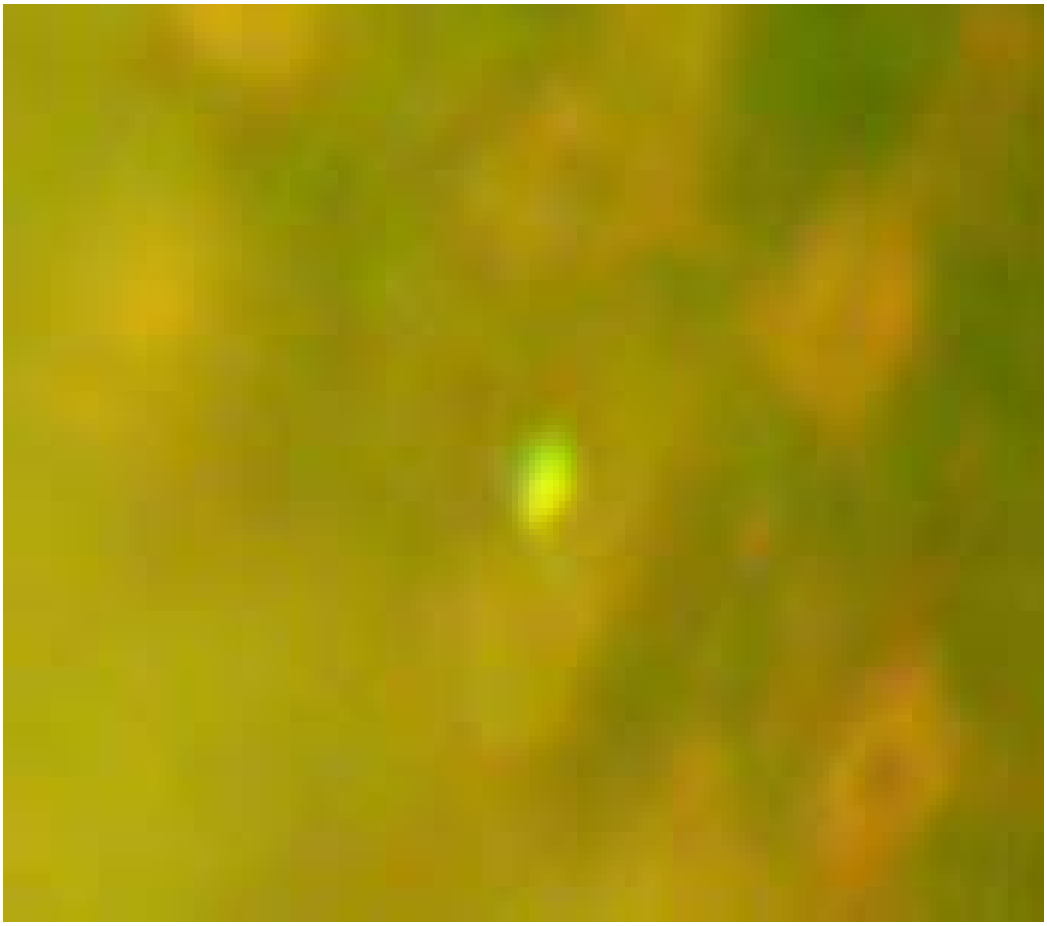


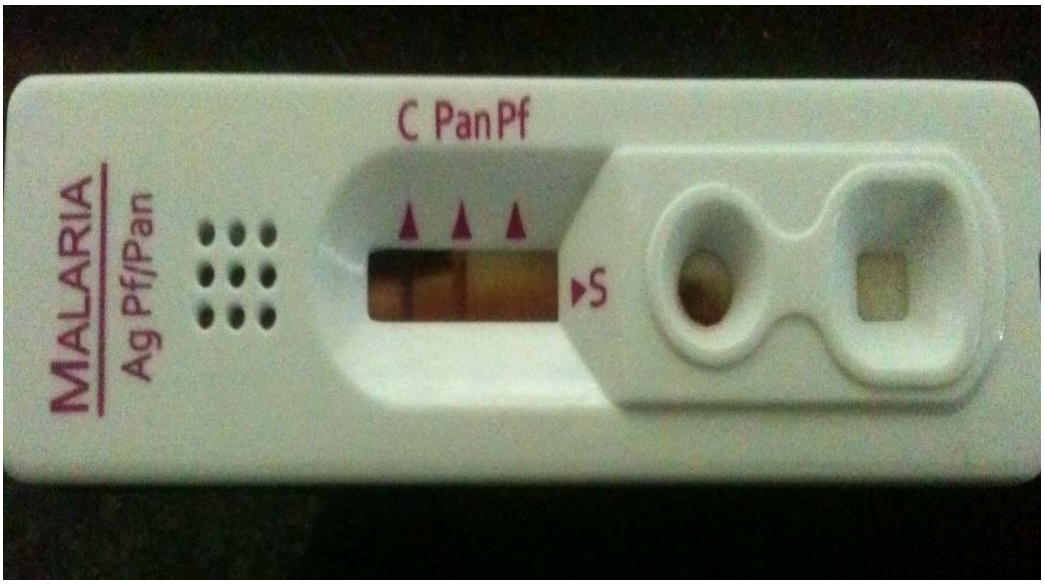
Figure : 17



RAPID CARD TEST

This test is a non-species specific test. According to the manufacture instruction, HRP band is to detect P.Falciparum and other band is for other type of plasmodium species. In the positive card test, Positve band was observed only in non-species specific band.Hence we concluded that it could be other than P.falciparum

Figure : 18



In our study among the 250 donors, one donor was found to be positive for malaria.

The below table shows the malaria positivity with the previous history of malarial infection (four months back). This shows that there was a statistically significant association by fisher's exact test (p-value-0.004)

Table : 9

Correlation between Donor with Previous History of Malaria and Serological Positivity

Past history of malaria			Malaria Seropositivity			Chi-Square	p- Value (FISHER'S Exact Test)
Present	Absent	Total	Positive	Negative	Total		
1	249	250	1	249	250	0.000	0.004

Table : 10

Correlation between Anti Malarial treatment and positivity for Malaria among VBD

Previous history of treatment for malaria			Positive Malaria		Total	Chi-Square	p-Value
Present	Absent	Total	Positive	Negative			
1	249	250	1	249	250	0.000	0.004

In the present study, one donor who had taken antimalarial drugs four months prior to the donation was found to be positive for malaria. Here also we found the significant association and the p value was 0.004.

SENSITIVITY AND SPECIFICITY OF QBC AND RDT WITH RESPECT TO GOLD STANDARD MICROSCOPY

Among the various tests for detection of malarial parasites, Microscopy has been considered as the Gold Standard method. In our study, QBC and RDT were compared with the gold standard microscopy.

Table: 11

Comparison of diagnostic test with Gold standard

QBC	MICROSCOPY		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	1	0	1
NEGATIVE	0	249	249
TOTAL	1	249	250

SENSITIVITY

$$= \frac{a}{a+c} \times 100$$

$$= \frac{1}{1} \times 100 = 100\%$$

SPECIFICITY

$$= \frac{d}{b+d} \times 100$$

$$= \frac{249}{249} \times 100 = 100\%$$

POSITIVE PREDICTIVE VALUE

$$= \frac{a}{a+b} \times 100$$

$$= \frac{1}{1} \times 100 = 100\%$$

NEGATIVE PREDICTIVE VALUE

$$= \frac{d}{c+d} \times 100$$

$$= \frac{249}{249} \times 100 = 100\%$$

Since we found only one positive among the entire test the Sensitivity and Specificity of the QBC were 100% with respect to the gold standard test microscopy.

Table : 12

Sensitivity and specificity of rapid diagnostic test with respect to microscopy

RDT	MICROSCOPY		TOTAL
	POSITIVE	NEGATIVE	
POSITIVE	1	0	1
NEGATIVE	0	249	249
TOTAL	1	249	250

SENSITIVITY

$$= \frac{a}{a+c} \times 100$$

$$= \frac{1}{1} \times 100 = 100\%$$

SPECIFICITY

$$= \frac{d}{b+d} \times 100$$

$$= \frac{249}{249} \times 100 = 100\%$$

POSITIVE PREDICTIVE VALUE

$$= \frac{a}{a+b} \times 100$$

$$= \frac{1}{1} \times 100 = 100\%$$

NEGATIVE PREDICTIVE VALUE

$$= \frac{d}{c+d} \times 100$$

$$= \frac{249}{249} \times 100 = 100\%$$

The Sensitivity and Specificity of the Rapid Diagnostic Test were 100% with respect to the Microscopy method. In the present study, sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy.

6. DISCUSSION

The present study screened 250 voluntary blood donors to know the prevalence of malaria and to compare the sensitivity of three different techniques (Microscopy, QBC, and RDT) for malarial screening. Voluntary blood donors with asymptomatic infection of malarial parasite contribute to the risk of Transfusion Transmitted Malaria.

In our blood bank, we strictly follow 100% voluntary blood donation. All the donors included in the study were voluntary blood donors. Hence the prevalence of TTI infection was very low in this study.

In our study out of 250 donors one was found to be positive for HBsAg and an another donor was found to be positive for malaria.

Gupta *et al* reported increased seropositivity of HIV, Anti HCV and HBsAg in replacement donors when compared to voluntary donors.[16]

Ekadashi *et al* from New Delhi, reported that Syphilis and HIV seropositivity were more among replacement donors when compared to voluntary donors.[98]

In the present study, the most common age group of voluntary blood donors were less than 22 years (30.4%) with overall sex distribution of 94% males and 6% females.

In the present study, among the 250 voluntary blood donors, one donor was positive for malaria by Microscopy, QBC and Antigen Detection Rapid Diagnostic Test. The prevalence rate of malaria in our donor study population was 0.4%.

Similar to our study, Bahadur *et al* in their study found malaria antigen prevalence rate of 0.03% among blood donors by immunochromatographic method. In their study, out of 11,736 units screened, three units were found positive for malarial antigen.

Among these three positive samples, 2 were positive for P.vivax and 1 was found to be positive for P.falciparum. These three cases were also found to be positive by microscopy.

Hence, they concluded that the use of rapid detection devices along with peripheral smear study of positive donor is a reliable method to prevent transfusion transmitted malaria in India.[8]

In concordance with our study, Choudry N *et al*. in their study conducted in northern India found the prevalence rate among voluntary blood donors to be 0.35% by antigen detection method. [58]

Lim CS *et al* in their study conducted in Korea among blood donors found the malarial antigen prevalence rate to be 1.7% by PCR method. In comparison to our study, the high antigen prevalence rate in their study was due to highly sensitive detection method.[57]

Saeed *et al* in their study conducted in Saudi Arabia found the prevalence rate among voluntary blood donors to be 0.17% by antigen detection ELISA method. This is similar to our study.[56]

Similar to our study, Diop *et al* in their study conducted in Senegal found that the prevalence rate among blood donors to be 0.53% by antigen detection ELISA method.[55]

Anju Dubey *et al* in their study in northern India reported that none of their donors were found positive by either Microscopy or antigen detection RDT. However, one of the donors who were deferred with history of malaria was found positive by antigen detection RDT and negative by microscopy, which accounts for 0.09% prevalence rate by antigen detection RDT among blood donors.

Therefore, they concluded that blood donor screening by Microscopy may not be an acceptable method, as more sensitive malaria screening methods like RDT, malaria antigen testing by ELISA are available.[47] This is similar to our study with respect to the past history of malaria and positive antigen detection RDT. However, in our study microscopy also found to be positive.

In the present study, sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy.

SC Parija *et al* in their study compared microscopy (gold standard), QBC and antigen detection test and reported the sensitivity and specificity of QBC were 78.94%

and 98% respectively while sensitivity and specificity of RDT were 75% and 100% respectively.[99]

Mishra *et al* in their study reported the sensitivity and specificity of RDT for detection of P.vivax was 100% and the sensitivity and specificity of RDT for detection of P.falciparum was 96% and 100% respectively when compared with microscopy as gold standard.[100]

In 2008, Bharti *et al* in their study at New Delhi evaluated the usefulness of new rapid diagnostic test (HRP2/ pLDH Malaria card test) for malaria diagnosis in the forested belt of central India. Their analysis revealed that in comparison to microscopy RDT was 93%sensitive, 85% specific with a positive predictive value of 79% and a Negative predictive value of 95%.[101]

Table : 13

The advantage and disadvantage of Microscopy are given below:

S.No	Advantage	Disadvantage
1	Gold Standard	It requires well trained microscopists
2	Inexpensive , when compared to other methods	Less sensitive
3	No need for any special instrumentation.	Time consuming
4	No storage or refrigeration required	60 – 120 mins
5	Non-Biodegradable substance are not used	It is Subjective

Table : 14

The advantage and disadvantage of QBC are given below:

S.No	Advantage	Disadvantage
1	Shorter duration (5 – 30 minutes)	It is expensive
2	High Sensitivity	Needed a special instrumentation
3	Highly reliable and user friendly	Needed a well trained and experience Microscopists
4	Different stages of parasite concentrated in a narrow zone can be viewed easily	Artifacts

Table : 15

The advantage and disadvantage of RDT are given below:

S.No	Advantage	Disadvantage
1	Easy to use with minimal training required	It is costlier than Microscopy method
2	Plasmodium species can be detected	Exposure to extreme temperature can result in poor performance of RDTs
3	The test can be performed without Laboratories	HRP2 alone kits detect only plasmodium falciparum
4	Time duration is less than 5 – 20 mins.	Intensity of the test band varies with amount of antigen present at low parasite densities. This leads to reader variation in test results

7. SUMMARY

This cross sectional study was conducted on 250 voluntary blood donor blood samples in Chennai, to find out the prevalence of malaria and to compare the sensitivity of three screening tests viz., Microscopy, QBC and RDT.

- The prevalence of malaria among the voluntary blood donors was 0.4%.
- Among 250 donors, one of the donor blood samples was found to be positive by Microscopy, QBC and RDT. The species identified was P.vivax.
- Malaria positive donor was observed in the age group of 22 years.
- The positive case was detected in the male gender.
- The infected donor was B blood group with Rh positive
- The sensitivity and specificity of QBC and RDT were 100% with respect to the gold standard microscopy.
- The positive asymptomatic donor had past history of malaria, for which he was treated with antimalarial drugs four months prior to blood donation.

8. CONCLUSION

In our study, among 250 voluntary blood donors in a malarial endemic area one sample was found positive by microscopy, Quantitative Buffy Coat Method and Rapid Diagnostic Test.

Since intermittent asymptomatic period, recrudescence and relapse are known with P.vivax infection in an endemic area, especially in semi-immune individuals, it is imperative to screen all donors by less-laborious, less time-consuming and more sensitive methods, irrespective of a strict donor questionnaire.

RDT in our study was found equally sensitive to QBC and microscopy. QBC is more sensitive; however it needs specialized instruments, higher technical expertise and is costlier compared to RDT.

Hence, to prevent transfusion transmitted malaria in endemic areas like India screening of malaria by RDT may be included along with microscopy. However, a study on large number of voluntary blood donors is necessary to arrive at a definitive conclusion.

Even technically superior PCR method can miss very low parasitemic load causing transfusion transmitted malaria, strict donor deferral criteria and antibody screening may be considered similar to non-endemic areas.

But, this would definitely lead to shortage of donor pool and more wastage of collected units. If necessary, antibody screening positive units can be subjected to pathogen inactivation or alternatively post-transfusion chemoprophylaxis may be tried.

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DONOR INFORMATION SHEET

MALARIAL PARASITES AMONG VOLUNATARY BLOOD DONORS

TO FIND THE PREVALENCE

TO EVALUATE DIFFERENT TECHNIQUES

AIM

.

- To study the prevalence of malaria among voluntary blood donors
- To compare the various diagnostic methods to screen malarial parasite among blood donors and
- To find out the most sensitive screening test to detect the parasite.

PROCEDURE

3ml of Blood will be taken at the time of donation in a separate test tube. Peripheral Blood Smear will be prepared immediately. Serum will be separated using Standard Blood Banking Method to do the other methods.

BENEFITS AND RISKS

- All the investigations are done free of cost.
- There is no risk for these investigations.

CONFIDENTIALITY

Your privacy will be protected in so for as permitted by law. Only your researcher and Ethical committee members will have access to the data collected during the study.

PARTICIPATION

Your participation in this study is voluntary and you are free to decide now or later whether to continue or discontinue from the study.

NAME OF THE DONOR:

SIGNATURE :

DATE :

CONSENT

I confirm that I read and understood the information about the above research study dated _____ and I received chance to ask the questions.

My participation in this study is voluntary and I know that I am free to withdraw from the study at any time, without giving any reason and without affecting of my legal rights.

I agree to this access. I know that my identification will not be revealed in any details that is released to third persons or published.

I agree not to restrict or interfere any data or results that are obtained from this study.

I agree to participate in this research study for the above listed purpose.

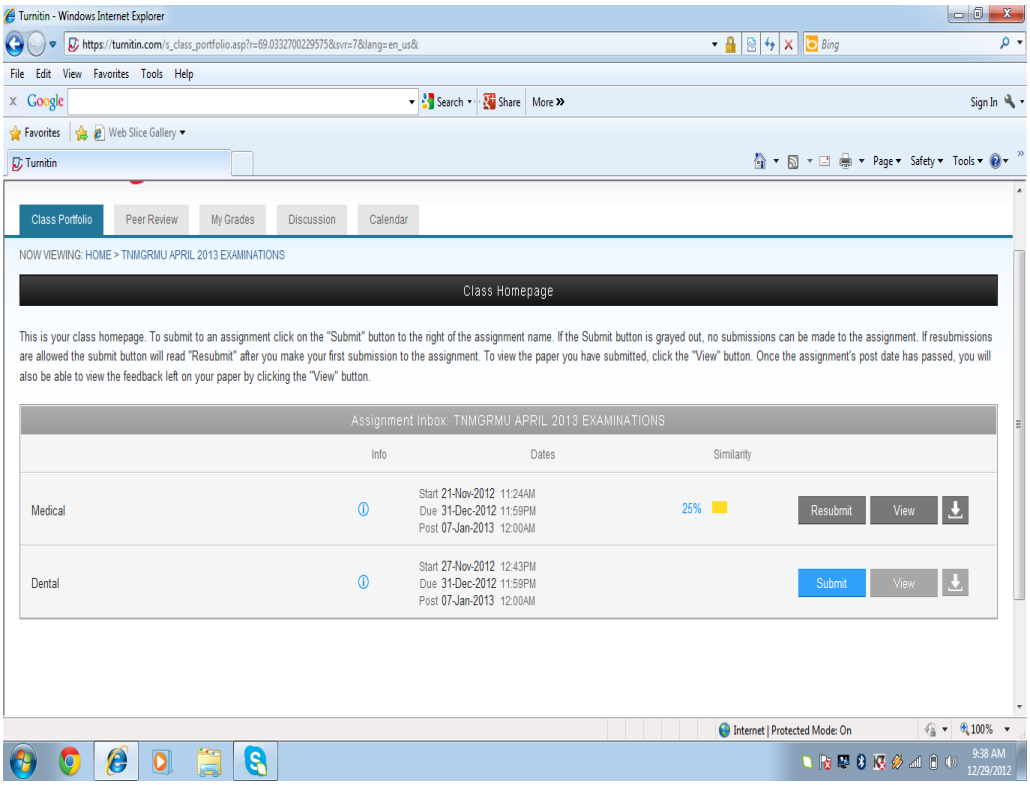
Donor's name :

Signature : Date :

Signature of the person

who obtains consent : Date :

Donor ID Number :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